theranos	Cystatin-C	Document Number: CL-RPT-145006 Revision: A
	Validation Document	Effective Date: 1/17/2014
Cystatin C Validation Report		

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1 ASSAY BACKGROUND

Cystatin C is a cysteine proteinase inhibitor with a relative molecular weight of 13,250 daltons and is formed by all nucleated cells investigated. Since it is formed at a constant rate and freely filtered by the healthy kidney, this protein is a good marker of renal function. Serum concentrations of cystatin C are almost totally dependent on the glomerular filtration rate. A reduction in the glomerular filtration rate (GFR) causes a rise in the concentration of cystatin C. Cystatin C has not been shown to be affected by factors such as muscle mass and nutrition, factors which have been demonstrated to affect creatinine values. In addition, a rise in creatinine does not become evident until the GFR has fallen by approximately 50%.

2 REGULATION AND GUIDANCE

2.1 The qualification/validation of the ELISA assays on the Theranos device will be in accordance with C.F.R. Ch IV, § 493.1253 "Standard: Establishment and verification of performance specifications" and outlined in CLSI guideline C28A3.

3 PRINCIPLE OF THE PROCEDURE

The Cystatin C (CYSC) latex reagent is a suspension of uniform latex particles coated with anti-cystatin-C antibody. When serum or plasma containing cystatin C is mixed with the latex reagent, agglutination takes place resulting in an increase in turbidity. This turbidity is measured at 571 and 805 nm. The cystatin C concentration in serum or plasma is determined from a calibration curve that is generated with the calibrators.

Plasma samples were diluted 1:3.125 fold in saline prior to analysis.

4 CALIBRATION

4.1 In 42 CFR Part 493.1255, it is required to perform calibration procedures with at least the frequency recommended by the manufacturer, or using criteria specified by the laboratory, or when calibration verification fails to meet acceptable limits.

4.1.1 The term "calibration verification," as used in CLIA, includes:

4.1.1.1 Confirming that a calibration meets the method manufacturer's specifications

4.1.1.2 Verifying that the calibration is suitable for the entire measuring interval (or "reportable range," which is the CLIA term)

4.2 Calibrators were diluted 1:3.125 and verified on the ADVIA system

4.2.1 This dilution factor is within the acceptable limits of the ADVIA internal calibration test.

4.3 For the purposes of this Validation Plan, calibration was carried out with every new lot of reagents.

4.3.1 Each level was tested in replicates of 3 and the average was used to create a standard curve for testing.

4.3.2 The calibration was verified using quality controls.

5 QUALITY CONTROL

5.1 Two to four level quality control samples, as appropriate to the assay, were analyzed with each calibration and before each test during the validation.

5.1.1 Low = .317 mg/L

5.1.2 Mid = .405 mg/L

5.1.3 High = .474 mg/L

5.2 The QC levels are not included when generating the calibration curve.

6 PRECISION

6.1 Precision was evaluated according to CLSI standard EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods. A total of 20 runs were performed over 10 days with 2 runs per day and 2 replicates per run for a total of 40 data points. The following tables indicate the between-run, between-day and within-laboratory precision at 3 levels:

Table 1: Precision at 3 medical decision limits

Precision

CLSI guideline EP05-A2 section 10.6

Level = L1

Number of observations	40
Number of runs	20
Number of days	10
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	0.339

	SD	95% CI	CV
Repeatability	0.009	0.007 to 0.013	2.7%
Between-run	0.008		2.4%
Between-day	0.000		0.0%
Within-laboratory	0.012	0.010 to 0.016	3.6%

Level = Level 2

Number of observations	40
Number of runs	20
Number of days	10
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	0.424

	SD	95% CI	CV
Repeatability	0.007	0.005 to 0.010	1.6%
Between-run	0.000		0.0%
Between-day	0.010		2.3%
Within-laboratory	0.012	0.009 to 0.018	2.8%

Level = Level 3

Number of observations	40
Number of runs	20
Number of days	10
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	0.507

	SD	95% CI	CV
Repeatability	0.009	0.007 to 0.013	1.7%
Between-run	0.000		0.0%
Between-day	0.013		2.6%
Within-laboratory	0.016	0.012 to 0.025	3.1%

6.2 Acceptance criteria:

Total allowable error (TAE %) of 28%, was selected as the acceptance criteria for this assay following proficiency guidelines recommended by the American Proficiency Institute Peer Data for

2013 CHEMISTRY / IMMUNOLOGY / IMMUNOHEM -1ST EVENT, using a cutoff of +/- 2SD. Allowable bias was calculated as the residual error budget after precision values (CV %) were subtracted from TAE (%).

Table II Total Allowable Error % (TAE%)

	Level 1	**Level 2**	**Level 3**
TAE%	28	28	28
CV (%)	3.6	2.8	3.1
Allowable Bias (%)	24.4	25.2	24.9
Bias (%)	0	0	0
Decision	Pass	Pass	Pass

7 BIAS ESTIMATION:, Predicate (Siemens) versus p-Assay (Theranos)

7.1 Twenty (20) venous samples were run using the predicate Siemens protocol without dilution, and in parallel on the Theranos assay with pre-dilution. Results were plotted in a scatter diagram, and a simple linear regression was performed (Figure I). Raw data as well as the scatter-plot summarizing the results are shown in Table III.

7.2 Mean bias comparing methods was calculated as follows: %Bias=[(Theranos-Siemens)/Siemens]*100 and results are shown in the column labelled "% difference" and indicated in Section 6.2.

7.3 Mean bias is less than allowable bias therefore, the acceptance criteria PASS.

Table III. doesn't match

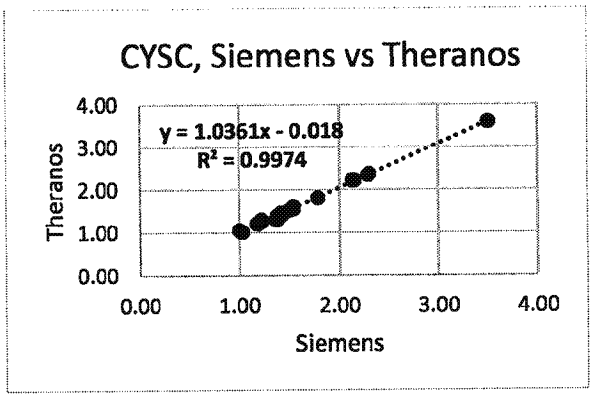

Figure 1

Table III Method comparison between predicate (Siemens) and Theranos p-assays

Sample #	Siemens	EDTA	Theranos	EDTA2	% difference
1	CYSC	0.57	T-CYSC	**0.55**	4%
2	CYSC	0.58	T-CYSC	0.57	2%
3	CYSC	0.65	T-CYSC	0.66	-2%
4	CYSC	0.66	T-CYSC	0.65	2%
5	CYSC	0.67	T-CYSC	0.68	-1%
6	CYSC	0.71	T-CYSC	0.72	-1%
7	CYSC	0.71	T-CYSC	0.69	3%
8	CYSC	0.72	T-CYSC	0.73	-1%
9	CYSC	0.73	T-CYSC	0.71	3%
10	CYSC	0.77	T-CYSC	0.76	1%
11	CYSC	0.80	T-CYSC	0.80	0%
12	CYSC	0.81	T-CYSC	0.81	0%
13	CYSC	0.82	T-CYSC	0.81	1%
14	CYSC	0.87	T-CYSC	0.85	2%
15	CYSC	0.87	T-CYSC	0.88	-1%
16	CYSC	0.92	T-CYSC	0.90	2%
17	CYSC	0.93	T-CYSC	0.95	-2%
18	CYSC	0.94	T-CYSC	0.95	-1%
19	CYSC	**0.98**	T-CYSC	**0.97**	1%
20	CYSC	**1.03**	T-CYSC	**1.05**	-2%
Average					**0%**

8 CTN REFERENCE RANGE VERIFICATION

8.1 20 unique fingerstick samples collected in capillary tube and nanotainers (CTNs) were collected from healthy donors and assayed in duplicate using the Theranos methods, as shown in Table III. Since predicate and Theranos results are closely correlated, no correction factor was applied.

8.2 17/20 (85%) of Theranos values fell within the predicate reference range (0.56-0.95 mg/L) (Table III, column 5), however, after excluding data points where the predicate assay also fell out of the reference range (Table III, column 3, bold), 17/18 (94.4%) of the Theranos values fell within the reference range. According to CLSI recommendations, (C28-A3c), 95% of values should fall within the reference range.

9 REFERENCES

9.1 Code of Federal Regulations, Title 42, Chapter IV, Subchapter G, Part 493, Subpart K, Sections 493.1217, 493.1253, and 493.1255.

9.2 DeSilva B, Smith W, Weiner R, et al. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharmaceutical Res. 2003; 20:1885-1900.

9.3 Guidance for Industry: bioanalytical method validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 2001.

9.4 R (version 2.13.1). The R Foundation for Statistical Computing, 07/08/2011.

9.5 StatisPro (version 1.13.00). Clinical and Laboratory and Standards Institute, Wayne, PA. 07/14/2011.

9.6 Dexter-Immunoassay (version1.0), Theranos, Inc., 2009.

9.7 EP10-A3, Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition, 2006, Clinical and Laboratory Standards Institute, Wayne, PA.

9.8 EP15-A2, User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition. 2005, Clinical and Laboratory Standards Institute, Wayne, PA.

9.9 EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (Interim Revision), 2010, Clinical and Laboratory Standards Institute, Wayne, PA.

9.10 EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.

9.11 EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2003, Clinical and Laboratory Standards Institute, Wayne, PA.

9.12 EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.

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